

## IN THE CLAIMS

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117. (New) Pulsed field electrophoresis chambers with Transversal Alternating Field Electrophoresis (TAFE) electrode array having two pairs of electrodes, each pair formed by one cathode and one anode, for separating DNA molecules immobilized in agarose plugs loaded into a vertical gel by means of using a system for energizing their electrodes and alternating the direction of application of an electric field crossing transversally the gel and generated by the electrode array, as well as a system for circulating a buffer solution throughout the chamber, which TAFE chambers comprise:

- i) one or various minigels placed in zones crossed by lines of force of the electric field interacting directly with DNA molecules loaded into said minigel(s), wherein said zones are Useful Electrophoresis Zones (UEZ) of said TAFE chambers,
- ii) pairs of electrodes of opposite polarities, cathode and anode, separated in TAFE electrode array a distance 'd', which is from 6.2 to 15 cm; wherein said separation in conjunction with the number and sizes of said UEZs limit to certain values height, depth and width of said TAFE chambers, length and width of said minigel(s), and total number of samples loaded simultaneously into all said minigel(s),
- iii) blocks of materials of high dielectric constant occluding zones of said TAFE chambers crossed by the electric field force lines not acting on DNA molecules loaded into said minigel(s), wherein said zones are non-useful electrophoresis zones (NEZ) zones of said TAFE chambers,

- iv) stretched electrodes, fixed and pulled tight by the action of a fixation and a tension system,
- v) inverted TAFE electrode configuration in said electrode array, and
- vi) three accessory sets of said TAFE chambers for homogenizing the electric current flowing through said chambers in the electrophoresis; the first set being formed by removable sheets that avoid turbulences of buffer circulated at high flow velocity; the second set comprising disassemblable sets of frames, base plates, covers and combs having teeth for casting said minigels with homogeneous cross sectional area and identical wells; and the third set comprising disassemblable systems of grooved blocks or sample plug makers, covers of said grooved blocks, and cutters for preparing DNA samples immobilized in plugs for said minigels.

118. (New) Electrophoresis chambers of claim 117 wherein each electrode of said electrode array is parallel to frontal wall of each TAFE chamber, up to 50 cm in length ('L'), and its length is equal to the width of said chamber.

119. (New) Electrophoresis chambers of claim 117 wherein said minigels are d

- 0.515 cm in length and said length is from 3.2 to 7.7 cm.

120. (New) Electrophoresis chambers of claim 117 wherein said minigels are from 1.7 to 50 cm in width have a number 'NM' of wells supporting 'NM' number

of DNA sample plugs, wherein said 'NM' is equal to (said minigel width – 0.2) / 0.25.

121. (New) Electrophoresis chambers of claim 117 wherein said distance 'd', or separation between opposite polarity electrodes, limits height and depth of said TAFE chambers by limiting to  $[2+1.4 \bullet d] \bullet [2 + 0.54 \bullet d] - 1.02 \bullet [1+ 0.54 \bullet d]^2 \text{ cm}^2$  the area of chamber lateral wall, or wall supporting the electrodes and the minigels, wherein said area is from 37.8 to 147.8  $\text{cm}^2$ .

122. (New) Electrophoresis chambers of claim 117 wherein said TAFE chambers are evenly subdivided in several UEZs with one minigel in each UEZ, wherein the number of UEZs is from 1 to 30.

123. (New) Electrophoresis chambers of claim 122 wherein said UEZs are as wide as each minigel and are fully occluded with rectangular blocks made of materials with high dielectric constant.

124. (New) Electrophoresis chambers of claim 117 wherein said minigels are arranged sequentially one next to the other, with their faces parallel to the electrodes, and widthwise said TAFE chambers.

125. (New) Electrophoresis chambers of claim 117 wherein the electrode array of one of said TAFE chambers is in fixed or removable single electrode platform (type I TAFE chamber), wherein each electrode of said electrode array is up to 50 cm in length.

126. (New) The electrophoresis chamber of claim 125 wherein said single electrode platform of said type I TAFE chamber is evenly subdivided and forms several UEZs with all minigels supported in a single frame.

127. (New) The electrophoresis chamber of claim 125 wherein said single electrode platform of said type I TAFE chamber is evenly subdivided and forms several UEZs with each minigel independently placed in each UEZ, wherein said minigels slide through laterally grooved pieces of said TAFE chambers.

128. (New) Electrophoresis chambers of claim 117 wherein said electrodes of one of said TAFE chambers are in various fixed or removable independent mini-platforms of electrode array, wherein each mini-platform limits one useful electrophoresis zone (UEZ), has one minigel, and bears its electrodes physically separated from the electrodes of the remaining mini-platforms (type II TAFE chamber); wherein said physically separated electrodes of one mini-platform acquire continuity with the electrodes of said remaining mini-platforms by plugging them in parallel, so, said TAFE chamber is energized with a single



power supply and samples loaded into the minigels of the mini-platforms are at the same electrophoresis conditions.

129. (New) Electrophoresis chambers of claim 117 wherein said inverted TAFE electrode configuration comprises the cathodes of the electrode array at the bottom of the electrophoresis chamber and the anodes at the top, so samples are loaded into the minigel bottom and migrate in the direction opposite to the gravity.

130. (New) Electrophoresis chambers of claim 117 wherein one of said blocks occluding NEZ of said TAFE chambers has its outer side parallel to the frontal wall of said TAFE chambers and its inner side forms a small angle with a plane containing cathode and anode located at the same side of the minigel(s).

131. (New) Electrophoresis chambers of claim 117 wherein said stretched electrodes are fixed to said TAFE chambers by the action of the fixation system, wherein said system comprises bored elastic plugs inserted into holes drilled into lateral walls of said chambers, and said electrodes enter into said TAFE chambers from the outside passing through the bores of said elastic plugs.

132. (New) Electrophoresis chambers of claim 131 wherein said elastic plugs are made of silicone or rubber.

133.- Electrophoresis chambers of claim 117 wherein said system for pulling tight each electrode of said TAFE chambers comprises

- i) a slotted rod with a hole crossing a waist-shaped notch, wherein said slotted rod bears the slot in its top side,
- ii) said electrode inserted into said hole, bent and wrapped around said waist-shaped notch,
- iii) a grub screw for immobilizing or releasing said slotted rod in desired position,

wherein said slotted rod is located at the electrode exit of said TAFE chamber.

134. (New) Electrophoresis chambers of claim 117 wherein said first set comprises two removable and identical sheets made of a material with high dielectric constant, wherein each sheet is the size of the chamber wall parallel to the electrodes and bears a horizontal slot in its third inferior part, wherein said slot is 0.3 cm in height and as long as each electrode.

135. (New) Electrophoresis chambers of claim 134 wherein the two identical sheets are placed as follows: one of said sheets near to the buffer inlet and the other near to the outlet, and said sheets divide said TAFE chambers in three compartments: a central compartment containing the electrodes and the

minigels, and two lateral compartments for delivering buffer solution into the chambers or withdrawing said buffer solution from them.

136. (New) Electrophoresis chambers of claim 117 wherein the second set of said TAFE chambers comprises:

- i) a flat base plate,
- ii) two frames with two lateral notches for inserting the combs, wherein said frames are from 0.35 to 0.5 cm in thickness and have rectangular or square shaped cavities determining the shape, thickness ('th'), length and width ('a') of each minigel cast in one of said frames,
- iii) a first comb, or comb with long teeth,
- iv) two covers: the first cover fitting against the front of said first comb, and the second cover fitting against the back of said first comb,
- v) a second comb, similar to said first comb, but with shorter teeth for pushing and aligning samples loaded into said minigels.

137. (New) Electrophoresis chambers of claim 136 wherein said first comb is flat in its frontal part, and thicker over the teeth, forming a step in the rear, wherein said teeth are identical and from 0.03 to 0.1 cm thick, from 0.15 cm to  $(a - 0.3)$  cm in width, and  $(th - 0.1)$  cm in length, wherein 'a' and 'th' are width and thickness of said minigel, respectively.

138. (New) Electrophoresis chambers of claim 136 wherein said second comb has shape and sizes similar to said first comb, excepting the length of the teeth, which are 0.2 cm shorter.

139. (New) Electrophoresis chambers of claim 136 wherein the second cover has two flat surfaces and a protruding edge; and the first cover has two flat surfaces, but one of its edges has a bevel cut in wedge formation.

140. (New) Electrophoresis chambers of claim 117 wherein the third set of said TAFE chambers, for preparing said DNA samples immobilized in plugs for minigels, comprises:

- i) various sample plug makers, wherein each sample plug marker is a flat impermeable block thicker than 0.5 cm with multiple parallel grooves lengthwise, wherein each groove is 0.2 cm in width and from 0.03 to 0.1 cm in depth, and said depth matches the thickness of teeth of a given comb of said second set,
- ii) a flat rigid and impermeable sheet of at least 0.1 cm in thickness, or cover of said sample plug maker,
- iii) sample plug cutters, wherein each cutter is a bar as long as, or longer than the grooves of one of said sample plug makers, and said bar bears several protuberances with cutting edges in its inferior part and protruding 0.1 cm

from the bar, wherein said cutting edges are evenly spaced, and said spacing is 0.15 cm at least.

141. (New) Electrophoresis chambers of claim 117 wherein one of said TAFE chambers bearing a single UEZ is energized at electric field strengths up to 25 V/cm using a power supply with maximum power output of 300 watt, provided 0.5X TBE buffer solution is used and maintained at constant temperature from 4 to 30°C, wherein 1X TBE is 89 mM Tris, 89 mM boric acid and 2 mM EDTA, pH 8.3.

142. (New) Electrophoresis chambers of claim 117 wherein one of said TAFE chambers with several UEZs is energized with a single power supply at electric field strength from 8 to 25 V/cm depending on the number of UEZs used, provided 0.5X TBE buffer solution is used and maintained at constant temperature from 4 to 30°C, wherein 1X TBE is 89 mM Tris, 89 mM boric acid and 2 mM EDTA, pH 8.3.

143. (New) A method for pulling tight electrodes in a Transversal Alternating Field Electrophoresis (TAFE) chamber having one or various Useful Electrophoresis Zones (UEZs), comprising the steps of:

- i) providing a system to pull tight each electrode of TAFE chambers, comprising

- a) a slotted rod with a hole crossing a waist-shaped notch,  
wherein said slotted rod bears the slot in its top side,
- b) said electrode inserted into said hole, bent and wrapped  
around said waist-shaped notch
- c) a grub screw for immobilizing or releasing said slotted rod in  
desired position
- ii) loosening said grub screw,
- iii) turning said slotted rod the required angle for pulling tight said  
electrode,
- iv) tightening said grub screw to set said slotted rod in the position  
that maintains said electrode stretched.

144. (New) A method for providing at least one minigel loaded with DNA sample plugs and presenting homogeneous cross sectional area to the ion flow during electrophoresis in a Transversal Alternating Field Electrophoresis (TAFE) chamber bearing one or multiples Useful Electrophoresis Zones (UEZs), comprising the steps of:

- i) providing
  - a) an accessory set, named second set, for casting minigels bearing homogeneous cross sectional area and identical wells, comprising at least:
    - 1. a flat base plate,

2. one frame bearing a rectangular or square shaped cavity and two lateral notches,
3. one first comb, or comb with long teeth bearing a step in its rear part over the teeth, and bearing teeth from 0.03 to 0.1 cm thick and at least 0.15 cm in width,
4. one first cover having two flat surfaces, but one of its edges bearing a bevel cut in wedge formation,
5. one second cover with a protruding edge fitting into said rear step of the first comb,
6. one second comb, or comb with short teeth,

b) an accessory set, named third set, comprising

1. at least one sample plug maker, or grooved block bearing lengthwise multiple parallel grooves of 0.03 to 0.1 cm deep,
2. a cover of sample plug maker,
3. at least one sample plug cutter having protuberances with cutting edges evenly spaced in 0.15 cm in its inferior part,

ii) assembling and casting in said second set at least one minigel bearing identical wells which are the sizes of the teeth of said first comb,

- iii) preparing identical DNA sample plugs in the sample plug maker bearing grooves which depth matches with the thickness of the teeth of the first comb used to cast the minigel,
- iv) placing DNA sample plugs on the wedge shaped edge of said first cover,
- v) pushing said DNA sample plugs for sliding them into said formed wells,
- vi) fitting the second comb into the notches of the frame and pushing said DNA sample plugs to the bottom of the wells for completely filling said wells.

145. (New) The method of claim 144 for assembling said second set and casting in it at least one minigel bearing identical wells, comprising the steps of:

- i) providing molten gel maintained between 65 and 70 °C,
- ii) placing said frame on said flat base plate,
- iii) fitting said first comb into said notches of said frame,
- iv) placing said first cover on said frame, in front of said first comb with the flat surface of the cover facing the frame and said bevel edge against said first comb
- v) clamping the set until interstices are sealed,
- vi) pouring said molten gel behind said first comb,



- vii) placing said second cover behind the first comb, and on the frame, introducing the protruding edge into the rear step of said first comb,
- viii) leaving the assembled accessory set until molten agarose solidified,
- ix) removing said first comb to leave wells formed in minigels.

146. (New) The method of claim 144 for assembling said third set and preparing in it DNA samples immobilized in identical plugs which are similar in sizes to the wells of the minigels cast in said second accessory set, comprising the steps of:

- i) providing an agarose cell suspension maintained at 45 °C,
- ii) pre-warming said grooved block and its cover at 45 °C,
- iii) pouring said agarose cell suspension into said grooves,
- iv) covering said grooved block with its cover and maintaining them at room temperature, or lower, until agarose solidifies,
- v) removing the cover of the sample plug maker,
- vi) aligning said sample plug cutter lengthwise on a groove of said grooved block with said protuberances and cutting edges turned to face said groove,
- vii) pressing down said sample plug cutter,
- viii) removing said sample plug cutter,
- ix) tilting said grooved block and pushing the plugs into a vessel, containing a solution to treat plugs,
- x) repeating steps (vi) to (ix) for all grooves of said grooved block.

147. (New) A method for varying, by occlusion, the number of Useful Electrophoresis Zones (UEZs) and volume of buffer solution required by a Transversal Alternating Field Electrophoresis (TAFE) chamber bearing multiple UEZs and minigels for performing electrophoresis of a number of DNA samples immobilized in plugs, comprising the steps of:

i) providing

a) a TAFE chamber comprising

1. an electrode array having TAFE electrode configuration, wherein electrodes of opposite polarities are separated a distance 'd' from 6.2 cm to 15 cm,
2. a total number of UEZs ( $N_{UEZtotal}$ ) and minigels comprised from 1 to 30,
3. a frontal wall parallel to electrodes and up to 50 cm in width ('L'),
4. various rectangular blocks made of a material with high dielectric constant and similar in shape to the UEZs for fully occluding said UEZs,

b) one minigel is from 1.7 to 50 cm in width and admits a number, named 'NM', of sample plugs equal to  $(\text{minigel width} - 0.2)/0.25$ ,

c) 'Nt' is said given number of DNA samples plugs in the electrophoresis,

- d) a buffer solution for performing electrophoresis,
- ii) calculating the number ('NUEZactive') of UEZs required for analyzing said total number of samples 'Nt', wherein said 'NUEZactive' is obtained from the formula ' $Nt = NM \times NUEZ_{active}$ ',
- iii) occluding unnecessary UEZ(s) by introducing into the chamber a number of said rectangular blocks equal to  $(NUEZ_{total} - NUEZ_{active})$ .
- iv) calculating said volume of buffer solution for filling said 'NUEZactive' in the chamber according to the formula  $[(2 + 1.4 \cdot d) \cdot (2 + 0.54 \cdot d) - 1.02 \cdot (1 + 0.54 \cdot d)^2] \cdot L \cdot NUEZ_{active} / NUEZ_{total}$ , wherein said volume is from 63.2 to 7390 ml and covers the minigels to a depth of 0.3 cm at least,

148. (New) A method for performing electrophoresis in a Transversal Alternating Field Electrophoresis (TAFE) chamber bearing from 1 to 30 Useful Electrophoresis Zones (UEZs), comprising the steps of:

- i) providing
  - a) a system for generating and switching an electric field,
  - b) a buffer solution,
  - c) a heat exchanger,
  - d) a given number of samples in the analysis,
  - e) a TAFE chamber bearing 1 to 30 UEZs comprising
    - 1. a first accessory set, for attenuating turbulences of buffer circulated at high flow velocity, comprising two removable

and identical sheets similar in size to frontal chamber wall  
and having a slot in the inferior part,

2. a second accessory set comprising at least one base plate,  
one frame, one comb of long teeth, one comb of short teeth,  
and two covers, for casting minigels and stamping wells in  
said minigels, wherein said wells are the size of said long  
teeth,
3. a third accessory set comprising at least one sample plug  
maker , a cover, and one sample plug cutter for preparing  
sample plugs, wherein said plugs are the size of the teeth of  
said comb of said second accessory set,

- ii) connecting said TAFE chamber to said system for generating and  
switching the electric field,
- iii) filling the chamber with said buffer solution,
- iv) connecting said TAFE chamber to said heat exchanger,
- v) checking the proper assembly of said first accessory set in said TAFE  
chamber,
- vi) circulating buffer solution throughout the chamber until a desired  
temperature is reached,
- vii) interrupting buffer circulation and placing vertically a minigel into each UEZ  
of said TAFE chamber,
- viii) restoring circulation at high flow velocity,

ix) energizing said TAFE chamber.

149 (New) The method of claim 148, further comprising prior to step (vii) the step of providing minigels loaded with DNA sample plugs.

150. (New) The method of claim 148, further comprising prior to step (iii) the step of varying, by occlusion, the number of UEZs and the volume of buffer solution required by the TAFE chamber for analyzing the given number of samples